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Effect of Long-term Reduction and Diel Fluctuation in Dissolved Oxygen on Spawning of Black Crappie, *Pomoxis nigromaculatus*

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ABSTRACT

Mature black crappies were exposed over winter in the laboratory to constant dissolved oxygen concentrations near 2.5, 4.0, 5.5, and 7.0 mg/liter. Starting on 26 April during a simulated spring-to-summer rise in water temperature, some were continued at the original oxygen concentrations while others were subjected to mean diel fluctuations ranging from 0.8 to 1.9 mg O_2/liter above and below the original concentrations. Controls were maintained at concentrations near air saturation. No spawning occurred at the lowest fluctuating treatment of 1.8 to 4.1 mg/liter. This treatment also caused behavioral aberrations as the water temperature reached 20 C. Successful spawning occurred during all other treatments.

Diel fluctuations of dissolved oxygen (DO) concentrations in aquatic habitats are often enhanced by organic pollutants. Although a large literature exists on the DO requirements of freshwater fish, the relationship between diel fluctuations in DO concentrations and fish spawning success is unknown. Such information is essential for the establishment of meaningful water-quality criteria. Exposure of fish for short periods of time to diurnal fluctuations in DO concentration, from relatively high to low levels, can cause behavioral aberrations, deformities, and death of larvae (Spoor 1977), impaired growth of juveniles (Fisher 1963; Stewart et al. 1967; Whitworth 1968) and behavioral aberrations and changes in several components of the blood in larger fish (Bouck and Ball 1965; Bouck 1972).

The objective of this laboratory study was to determine the spawning success of the black crappie, *Pomoxis nigromaculatus*, exposed to diel fluctuations of DO concentration after exposure over winter to one of four constant reduced DO regimes. Black crappie spawning success was unaffected by 2-mo exposure to several constant reduced DO concentrations ranging from 2.5 to 6.6 mg/liter (Siefert and Herman 1977), although the time of first spawning appeared to be advanced at the lower concentration. Brungs (1971) found that fathead minnow *Pimephales promelas* spawning was inhibited at a constant 11-mo exposure to 1 mg O_2/liter and was reduced at 2 mg/liter; resulting larvae died within 2–5 days after hatching. Survival of larvae was reduced at concentrations up to and including 4 mg/liter. To our knowledge no other work has been reported on the effects of experimental long-term reductions in DO concentration on fish spawning success.

METHODS

The test apparatus, black crappie culture and rearing techniques, and chemical characteristics of the water supply have been described previously (Siefert and Herman 1977).

Black crappies were obtained from the Mississippi River near Genoa, Wisconsin, and transported to the laboratory in Duluth, Minnesota, on 30 September and 7 October 1976 at 13 C. Thereafter they were provided with Lake Superior water and were subjected to a simulated seasonal decrease in water temperature to an overwinter temperature of 4.5–7 C. On 26 April 1977 a simulated spring rise in temperature was initiated; at the end of the test on 1 July 1977 the temperature reached 23 C. The natural light cycle of Duluth, Minnesota was simulated.

To obtain the desired DO concentrations degassed water was mixed with air-saturated water in glass mixing chambers. This water was drawn by in-line pumps through four-way valves that were timer-controlled

and let water with the prescribed DO concentration into the system. It was then pumped through stainless steel coils in a water bath to reach desired temperatures and delivered into each test tank through flow meters at 1.1 liters/min. Control water was delivered in a thin layer down a cylindrical screen to eliminate supersaturation of gases.

On 9 November at a water temperature of 8 C, fish ranging from 33 to 475 g were graded into three size groups and distributed one at a time to 10 duplicate tanks until each contained 16 fish. Each tank received some fish from each size group. The 1.2-m diameter fiberglass tanks each contained approximately 734 liters of water maintained at near air saturation. Two days later the DO concentrations were gradually lowered to constant levels preset at about 7.0, 5.5, 4.0, and 2.5 mg/liter. Two tanks (controls) retained DO concentrations near or at air saturation. On 23 March five to seven of the fish in each tank at reduced DO concentrations were removed and placed in additional duplicate tanks; those transferred remained at the constant DO concentrations until the end of the test. Because of disease problems and to increase the probability of completing the following aspect of the study, the most healthy appearing and generally larger fish were left in the original tanks and were exposed to diel fluctuations in DO concentrations (lows at 0600 h and highs at 1800 h) of approximately 1.5 mg/liter above and below the constant levels (Fig. 1). This treatment was started on 26 April and corresponded with the initiation of the rise in water temperature.

Two artificial nests (Siefert and Herman 1977) were placed in each tank on 12 May when water temperatures reached 11 C. These nests were checked daily for embryos. If embryos were present and no spawning activity was evident, the nests were removed and the contents of each nest were considered a separate spawning. Two hundred embryos from a spawning were incubated and used for making "hatching" and "survival to swim-up" determinations. Each sample was cultured in two 100-ml screened-bottom vials suspended in the tank where spawned. After hatching in the sample was completed, unfertilized eggs, dead embryos, and deformed larvae were removed, and the remaining embryos were reared to swim-up. After sampling, the remainder of each spawn was measured volumetrically and then discarded.

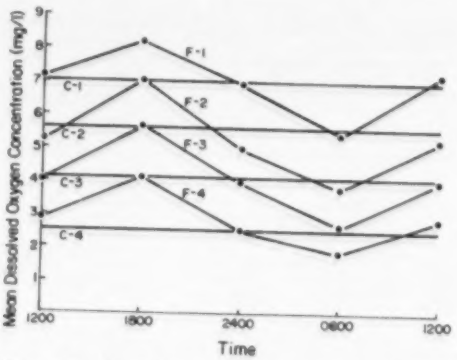

FIGURE 1.—*Mean dissolved oxygen concentrations for the four constant (C) and four diel fluctuating (F) treatments, 26 April to 1 July.*

The fish were fed to satiation on live fathead minnows three times a week until 23 May and daily thereafter. The fathead minnows were routinely treated with one or more 1-h prophylactic formalin treatments (250 mg/liter) before being used as food.

Dissolved oxygen concentrations were measured daily by the unmodified Winkler method in at least one of the duplicate tanks under control and constant reduced DO conditions and in all tanks under fluctuating conditions. In addition, the diel DO concentrations were monitored continuously with eight polarographic oxygen meters in conjunction with a multichannel electronic recorder. All data pertaining to oxygen concentrations are presented in Table 1. Total ammonia concentrations, which ranged from 0.0 to 0.42 mg/liter, were determined on five water samples from each experimental tank by the direct nesslerization method. At water temperatures from 6 to 20 C and pH from 7.2 to 7.7 the highest calculated un-ionized ammonia concentration was 0.003 mg/liter (Trussell 1972). Temperatures were continuously monitored in all tanks.

TABLE 1.—*Reproductive success of black crappies exposed during the winter months to several constant lowered dissolved oxygen concentrations and then either held at the constant level or subjected to diel fluctuations in concentration during a simulated spring-to-summer rise in water temperature.*

	Dissolved oxygen concentration (mg/liter) (mean ± standard deviations; ranges in parentheses)		Number of mature fish (male: female)	Number of spawnings
	Constant	Diel fluctuation[a] Low (0600 h)–high (1800 h)		
	11-11-76 to 7-1-77			
(1)	10.9 ± 1.5 (7.7–12.6)[c]		2:1	3
(2)	10.8 ± 1.4 (7.7–12.5)[c]		2:3	2
(3)	6.9 ± 0.3 (6.1–8.1)[c]		3:1[b]	7
(4)	5.5 ± 0.2 (4.7–6.2)[c]		2:1[b]	4
(5)	4.0 ± 0.4 (2.7–6.0)[c]		2:2[b]	6
(6)	4.2 ± 0.6 (3.1–7.6)[c]		1:1[b]	1
(7)	2.6 ± 0.2 (1.7–3.4)[c]		1:2[b]	3
	11-11-76 to 4-26-77	4-26-77 to 7-1-77		
(8)	6.9 ± 0.2 (6.4–7.2)[d]	5.4 ± 0.5 (4.3–6.4)–8.1 ± 0.8 (7.1–10.1)	4:2	6
(9)	7.2 ± 0.2 (6.5–9.3)[d]	5.6 ± 0.5 (4.7–6.6)–8.3 ± 0.7 (6.8–10.1)	2:3	6
(10)	5.5 ± 0.3 (4.9–6.1)[d]	3.6 ± 0.6 (2.2–6.0)–7.0 ± 0.8 (5.6–10.1)	4:4	14
(11)	5.5 ± 0.2 (4.2–6.0)[d]	3.9 ± 0.6 (2.5–5.0)–7.1 ± 0.7 (5.4–9.8)	4:2	3
(12)	4.0 ± 0.2 (3.3–5.1)[d]	2.7 ± 0.6 (1.9–4.4)–5.7 ± 0.8 (4.3–8.7)	3:2	15
(13)	4.0 ± 0.3 (3.3–5.1)[d]	2.6 ± 0.5 (2.0–3.9)–5.6 ± 0.8 (4.0–8.7)	4:1	0
(14)	2.6 ± 0.2 (1.9–3.4)[d]	1.8 ± 0.4 (1.0–2.6)–4.1 ± 0.6 (2.8–5.2)	3:3	0

Shortly after the initiation of the test and at cold temperatures, chronic infections of the protozoan *Ichthyophthirius multifiliis* and the bacterium *Aeromonas liquefaciens* were apparent in all tanks. Intermittent prophylactic treatments with Aureomycin[1] (20 mg/liter), Furnance[1] (0.2–0.5 mg/liter), Terramycin[1] (40 mg/liter), benzalkonium chloride (20 mg/liter), formalin (170–250 mg/liter), and a mixture of formalin (15 mg/liter) and malachite green (0.1 mg/liter) were ineffective in controlling the epizootics. On 9 May a 4-day treatment with a mixture of formalin (25 mg/liter) and malachite green (0.1 mg/liter) was initiated, which eliminated the protozoan infections. The fish were treated for 2, 3, 4, and 5 h at water temperatures ranging from 10 to 12 C. Water flows were maintained, and after the prescribed times the tanks were drawn down to 160 liters. Deaths and symptoms of the bacterial infections were finally eliminated by injecting for 3 wk 0.25 ml of Terramycin (5 mg active Terramycin in distilled water) into each fathead minnow immediately prior to its use as food. Disease-caused mortality was highest in the control tanks, 74% in each, and ranged from 25 to 44% of the original 16 fish in each tank at the reduced DO treatments.

A sufficient number of mature fish survived to provide spawning data from at least one tank at all constant and fluctuating DO treatment levels. Since a nesting male was not present in one control tank, on 16 June a mature male was transferred to this tank from the other control.

RESULTS AND DISCUSSION

Territorial behavior of the black crappie males was evident in most tanks on 23 May, and the first spawning occurred the next day at the constant DO concentration of 5.5 mg/liter and 14 C. Thereafter and at temperatures ranging from 15 to 21 C spawning occurred in the controls and at all constant reduced DO concentrations (means ranging from 2.5 to 6.9 mg/liter) and mean diel fluctuating concentrations of 2.7 to 5.7 mg/liter and above (Table 1). No sign of earlier spawning was evident at the reduced DO concentrations as was reported by Siefert and Herman (1977), but spawning success and reproductive behavior were similar to those of the previous study. The number of spawnings in each tank ranged from one to 15, producing embryo volumes ranging from

[1] The U.S. Environmental Protection Agency neither recommends nor endorses any commercial product. Trade names are used only for identification.

TABLE 1.—*Continued.*

	Spawning temperature (C)	Volume of embryos per spawning[b] (ml)		Percent hatched		Percent normal larvae surviving to swim-up	
		Mean[c]	Range	Mean[c]	Range	Mean	Range
(1)	15–19	83.0	73–96	87.0	81.0–96.5	75.8 (3)[e]	71.5–82.0
(2)	19–20[f]	58.5	58–59	66.3	48.0–84.5	54.8 (2)	34.0–75.5
(3)	16–20	18.7	5–32	72.1	42.5–90.0	72.9 (5)	55.0–87.0
(4)	14–18	34.5	28–48	79.3	74.0–88.0	70.1 (4)	62.0–83.5
(5)	16–18	20.0	15–25	80.0	66.0–85.0	61.8 (6)	34.0–79.0
(6)	17	6.0	6.0	21.0	21.0	17.0 (1)	17.0
(7)	16–20	36.7	16–64	84.8	73.5–94.5	74.9 (3)	60.0–85.7
(8)	15–20	40.0	18–79	67.8	47.0–95.0	58.6 (6)	35.5–83.5
(9)	16–20	19.8	6–28	75.5	48.0–85.5	63.0 (6)	42.8–73.0
(10)	15–18	33.5	5–82	76.4	38.5–90.0	64.8 (13)	26.0–87.0
(11)	15–20	69.0	65–74	71.0	50.5–92.5	57.0 (3)	27.0–85.5
(12)	15–21	23.1	8–46	66.5	24.0–96.0	57.3 (13)	27.0–90.5
(13)							
(14)							

[a] *N* ranged from 59 to 65.
[b] The mean ± standard deviation and range of the number of embryos per ml of 15 spawn samples was 811.5 ± 105.4 and 600–1,010, respectively.
[c] *N* is the number of spawnings.
[d] *N* ranged from 150 to 155. These data are from the tanks where the water was near air-saturation DO concentrations and are considered the controls.
[e] *N* is in parentheses.
[f] There were no mature males in this tank at lower water temperatures. A mature male was transferred from the other control at 19 C on 6-16-77.
[g] *N* ranged from 119 to 143.
[h] There were 1 or 2 immature fish in these tanks at test end.
[i] *N* ranged from 84 to 95.

5 to 96 ml. The mean volumes of embryos per spawning produced in nine of 10 tanks with reduced DO levels fell below the range of the mean values for the controls. We believe these differences were caused by many factors such as the mating of more than one female with a male at the same time, the spawning of one or more females with two nesting males, or differences in size (final weights 125–447 g) and number of females. In the controls the mean survival of embryos to hatch ranged from 66.3 to 87.0%, and that of resulting normal swim-up larvae from 54.8 to 75.8%. In nine of the 10 tanks at the reduced DO regimes survival of embryos and swim-up larvae were within these ranges.

The fish did not spawn at the lowest diel fluctuating treatment (1.8–4.1 mg DO/liter), although at the end of the test gonadal development in the two males and three females at this treatment appeared similar to that in the control fish. Testes of the males were examined microscopically and contained motile sperm. Feeding and male nesting behavior of these fish (also of four males in a duplicate exposure) appeared normal until 20 C was reached on 23 June. On this day and for several days thereafter some of the fish were seen near the water surface or breaking the surface with their mouths, and at times swimming in an erratic darting manner about the tank. The frequency of gill ventilation was increased in comparison to that of the fish at higher DO concentrations; also, the larger fish were lighter in color. These abnormalities were observed in the morning and coincided with the lowest portion of the diel fluctations in DO concentration. Later in the day as the DO concentration increased the fish seemed to recover from this stressed state. Similar abnormalities caused by diurnal fluctuating DO concentrations to about 3 mg/liter have been reported for several other centrarchids: bluegill (*Lepomis macrochirus*); large-

mouth bass (*Micropterus salmoides*); and rock bass (*Ambloplites rupestris*) (Bouck and Ball 1965; Bouck 1972).

Spawning did not occur among four males and one female in one tank with a mean diel fluctuation of 2.6–5.6 mg DO/liter, but was apparently uninhibited (15 spawnings) under similar conditions in the duplicate tank (mean diel fluctuation 2.7–5.7 mg/liter) containing three males and two females. No behavioral deviations of the fish were seen in these treatments.

The selection of the healthy appearing and generally larger fish for exposure to the fluctuating DO treatments should not have biased the results. At the end of the test, mature males at the constant treatments were smaller (mean weight 167.6 g; range 70–265 g) than those at the fluctuating treatments (mean weight 226.5 g; range 156–492 g). However, small and large males were observed defending nests and spawning. There was little difference among the average weights of the mature females. Mean weights in the constant and fluctuating treatments were 184.3 g (range 125–276 g) and 196.1 g (range 125–447 g), respectively. We also believe the number of females (three, ranging from 200–224 g) at the lowest fluctuating DO treatment was adequate for determining the effects on spawning because spawning occurred in 12 of 13 tanks containing from one to four females.

This study indicates that dissolved oxygen concentrations near 2.6 mg/liter or above are sufficient to provide the DO requirements of mature black crappie prior to (overwinter) and during the spawning period; and that diel fluctuations below this concentration to about 1.8 mg/liter during the spring water temperature rises can cause hypoxic stress and inhibit reproduction.

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REFERENCES

BOUCK, G. R. 1972. Effects of diurnal hypoxia on electrophoretic protein fractions and other health parameters of rock bass *Ambloplites rupestris*. Trans. Am. Fish. Soc. 101:448–493.

———, AND R. C. BALL. 1965. Influence of a diurnal oxygen pulse on fish serum proteins. Trans. Am. Fish. Soc. 94:363–370.

BRUNGS, W. A. 1971. Chronic effects of low dissolved oxygen concentrations on the fathead minnow *Pimephales promelas*. J. Fish. Res. Board Can. 28:1119-1123.

FISHER, R. M. 1963. Influence of oxygen concentration and of its diurnal fluctuation on the growth of juvenile coho salmon. M.Sc. Thesis. Oregon State Univ., Corvallis. 48 pp.

SIEFERT, R. E., AND L. J. HERMAN. 1977. Spawning success of the black crappie *Pomoxis nigromaculatus* at reduced dissolved oxygen concentrations. Trans. Am. Fish. Soc. 106:376–378.

SPOOR, W. A. 1977. Oxygen requirements of embryos and larvae of the largemouth bass, *Micropterus salmoides* (Lacépède). J. Fish Biol. 11:77–86.

STEWART, N. E., D. L. SHUMWAY, AND P. DOUDOROFF. 1967. Influence of oxygen concentration on the growth of juvenile largemouth bass. J. Fish. Res. Board Can. 24:475–494.

TRUSSELL, R. P. 1972. The percent un-ionized ammonia in aqueous ammonia solutions at different pH levels and temperatures. J. Fish. Res. Board Can. 29:1505–1507.

WHITWORTH, W. R. 1968. Effects of diurnal fluctuations of dissolved oxygen on growth of brook trout. J. Fish. Res. Board Can. 25:579–584.